

FORM PTO-1390 (Modified) (REV 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	
<b>TRANSMITTAL LETTER TO THE UNITED STATES</b> <b>DESIGNATED/ELECTED OFFICE (DO/EO/US)</b> <b>CONCERNING A FILING UNDER 35 U.S.C. 371</b>		ATTORNEY'S DOCKET NUMBER <b>101195-73</b>	
INTERNATIONAL APPLICATION NO <b>PCT/DE00/03104</b>		INTERNATIONAL FILING DATE <b>7 September 2000</b>	
U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR <b>10/069896</b>		PRIORITY DATE CLAIMED <b>16 September 1999</b>	
TITLE OF INVENTION <b>Agents for Treating Human Diseases, Especially for Treating Tumors Such as Colonic Cancers and Melanomas or for Regenerating Tissue and Promoting Hair Growth</b>			
APPLICANT(S) FOR DO/EO/US <b>Walter Birchmeier; and Jens-Peter von Kries</b>			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: <ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.</li> <li>4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31).</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).             <ol style="list-style-type: none"> <li>a. <input checked="" type="checkbox"/> is attached hereto.</li> <li>b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4).</li> </ol> </li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).</li> <li>b. <input type="checkbox"/> have been communicated by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).</li> <li>10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> <li>11. <input type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409).</li> <li>12. <input type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> </ol> <p><b>Items 13 to 20 below concern document(s) or information included:</b></p> <ol style="list-style-type: none"> <li>13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>15. <input checked="" type="checkbox"/> A <b>FIRST</b> preliminary amendment.</li> <li>16. <input type="checkbox"/> A <b>SECOND</b> or <b>SUBSEQUENT</b> preliminary amendment.</li> <li>17. <input type="checkbox"/> A substitute specification.</li> <li>18. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>19. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.</li> <li>20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4).</li> <li>21. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).</li> <li>22. <input checked="" type="checkbox"/> Certificate of Mailing by Express Mail</li> <li>23. <input type="checkbox"/> Other items or information:</li> </ol>			

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.101) <div style="font-size: 24pt; font-weight: bold; margin-top: 5px;">10/069896</div>	INTERNATIONAL APPLICATION NO. <div style="font-weight: bold; margin-top: 5px;">PCT/DE00/03104</div>	ATTORNEY'S DOCKET NUMBER <div style="font-weight: bold; margin-top: 5px;">101195-73</div>
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24. The following fees are submitted:

**BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5)) :**

☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO . . . . . **\$1040.00**

☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . . . . . **\$890.00**

☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . . . **\$740.00**

☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) . . . . . **\$710.00**

☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) . . . . . **\$100.00**

**ENTER APPROPRIATE BASIC FEE AMOUNT =**  

**\$890.00**

CALCULATIONS PTO USE ONLY

Surcharge of **\$130.00** for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). ☐ 20    ☒ 30

**\$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	9 - 20 =	0	x \$18.00		<b>\$0.00</b>
Independent claims	6 - 3 =	3	x \$84.00		<b>\$252.00</b>
Multiple Dependent Claims (check if applicable). <span style="float: right;"><input type="checkbox"/></span>					<b>\$0.00</b>
<b>TOTAL OF ABOVE CALCULATIONS =</b>					<b>\$1,272.00</b>
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.					<b>\$636.00</b>
<b>SUBTOTAL =</b>					<b>\$636.00</b>

Processing fee of **\$130.00** for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). ☐ 20    ☐ 30    +

**\$0.00**

**TOTAL NATIONAL FEE =**

**\$636.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☐

**\$0.00**

**TOTAL FEES ENCLOSED =**

**\$636.00**

	Amount to be: refunded	\$
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a. ☐ A check in the amount of \_\_\_\_\_ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 14-1263 in the amount of \$636.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1263 A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Correspondence address associated with Customer No.27387

27387

PATENT TRADEMARK OFFICE

Bruce S. Londa

NAME

33,531

REGISTRATION NUMBER

February 27, 2002

DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
Atty's Docket No. 101195-73

APPLICANT : Walter Birchmeier et al.  
FILED : Concurrently Herewith  
FOR : Agents for Treating Human Diseases, Especially  
for Treating Tumors Such as Colonic Cancers and  
Melanomas or for Regenerating Tissue and  
Promoting Hair Growth

PRELIMINARY AMENDMENT

Hon. Assistant Commissioner of Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please amend the application as  
follows:

**IN THE CLAIMS**

Please amend the claims as follows. Claims 4 and 9 are  
amended. A marked-up copy of the amended claims is also  
enclosed.

1. Agents for treating human disease, contained substances,  
which prevent the binding of  $\beta$ -catenin at LEF-1/TCF-  
transcriptionsfactors, APC or conductin/axin.
2. Method for detecting substances preventing binding of  $\beta$ -  
catenin selectively with LEF-1/TCF transcription factors,  
APC or conductin/axin wherein in the  $\beta$ -catenin molecule in



Cys 429, Asn 430, Asn 431, Asn 434, Met 437, Val 438) was identified in the vicinity of the LEF-1/TCF binding site,

- the compounds according to the annexed "drug list" and positive list" which thus become a subject of the claim are fitted into the identified hydrophobic pocket, subsequently experimentally checked with ELISA for their inhibition of the complex formation of  $\beta$ -catenin and its binding partners and optimized by chemical modification.

5. Substances with a lead structure essential for binding in the hydrophobic pocket and inhibition of the formation of complexes with  $\beta$ -catenin marked by cephalosporines of the cefamandole type (molecule class IA), with the lead structure essential for binding in the hydrophobic pocket and inhibition of the formation of complexes with  $\beta$ -catenin consisting of an aromatic ring, an organic component similar to the structure of the ala-ala dipeptide and a  $\beta$ -lactam and thiazole ring, which are preferably cephalosporines of the cefamandole type such as e.g. cefsulodine, cefadroxil or cefamandole nafates.

6. Substances for the inhibition of the complex formation of  $\beta$ -catenin with LEF/TCF marked by a structure corresponding to AC-(6-0-stearoyl)-muramyl-ala-D-isoglutamine (molecule class IB) .

7. Substances for the inhibition of the complex formation of  $\beta$ -catenin with LEF/TCF marked by a structure corresponding to that of 3,6-dihydroxybenzonobornane (molecule class IC).

8. Substances binding in the hydrophobic pocket without inhibiting the formation of complexes, however affecting

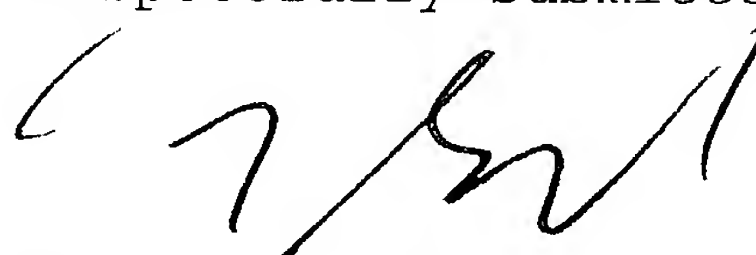
binding of cefamandole to  $\beta$ -catenin as guiding structures for developing potent inhibitors, preferably substances of the «positive list» table which thus becomes an object of the claim.

9. Agents according to claim 1 for treating tumors, such as colonic cancers and melanomas, or for regenerating and promoting hair growth.

#### REMARKS

The above amendments were made to place the application into proper United States Patent Format.

Respectfully Submitted,




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# Amended Claims - Marked-Up Pages

1. Agents for treating human disease, contained substances, which prevent the binding of  $\beta$ -catenin at LEF-1/TCF-transcriptionsfactors, APC or conductin/axin.
2. Method for detecting substances preventing binding of  $\beta$ -catenin selectively with LEF-1/TCF transcription factors, APC or conductin/axin wherein in the  $\beta$ -catenin molecule in the vicinity of essential binding sites hydrophobic pockets are identified and subsequently therapeutic substances fitting into this pocket are synthesised and tested.
3. Method according to claim 2 wherein  $\beta$ -catenin mutants were identified which mark the respective essential binding site for LEF-1/TCF transcription factors, APC or conductin,
  - the surfaces in this region are calculated based on X-ray crystallographic analytical data of the armadillo region, thus identifying the existing hydrophobic pockets,
  - low-molecular compounds are fitted in this pocket and are selected in a biological assay owing to their stabilizing interactions with  $\beta$ -catenin and their selective inhibition or promotion of complex formation with LEF/TCF, APC or conductin and
  - these compounds are further modified, if necessary by adding acid groups.
4. (amended) Method according to ~~claims 2 and 3~~ claim 2,  
wherein  
the  $\beta$ -catenin-mutants Lys 435, Arg 469, His470, Lys 508 Arg 515, which mark the essential binding site for LEF-1



- the  $\beta$ -catenin-mutants Phe 235, His 260, Lys 292 which mark the essential binding site for conductin,
  - the  $\beta$ -catenin-mutants Lys 345, Trp 383, Arg 386, which mark the essential binding site for APC  
were identified,
  - the molecule surface is calculated with the aid of the programs Grasp, Ludi and similar programs,
  - a hydrophobic pocket (flanked by the amino acids Val 358, Met 363, Ala 391, Ala 392, Thr 393, Lys 394, Gln 395, Met 398, Leu 401, Leu 402, Ile 423, Asn 426, Leu 427, Thr 428, Cys 429, Asn 430, Asn 431, Asn 434, Met 437, Val 438) was identified in the vicinity of the LEF-1/TCF binding site,
  - the compounds according to the annexed "drug list" and positive list" which thus become a subject of the claim are fitted into the identified hydrophobic pocket, subsequently experimentally checked with ELISA for their inhibition of the complex formation of  $\beta$ -catenin and its binding partners and optimized by chemical modification.
5. Substances with a lead structure essential for binding in the hydrophobic pocket and inhibition of the formation of complexes with  $\beta$ -catenin marked by cephalosporines of the cefamandole type (molecule class IA), with the lead structure essential for binding in the hydrophobic pocket and inhibition of the formation of complexes with  $\beta$ -catenin consisting of an aromatic ring, an organic component similar to the structure of the ala-ala dipeptide and a  $\beta$ -lactam and thiazole ring, which are preferably cephalosporines of the cefamandole type such as e.g. cefsulodine, cefadroxil or cefamandole nafates.



6. Substances for the inhibition of the complex formation of  $\beta$ -catenin with LEF/TCF marked by a structure corresponding to AC-(6-0-stearoyl)-muramyl-ala-D-isoglutamine (molecule class IB) .
7. Substances for the inhibition of the complex formation of  $\beta$ -catenin with LEF/TCF marked by a structure corresponding to that of 3,6-dihydroxybenzonobornane (molecule class IC) .
8. Substances binding in the hydrophobic pocket without inhibiting the formation of complexes, however affecting binding of cefamandole to  $\beta$ -catenin as guiding structures for developing potent inhibitors, preferably substances of the «positive list» table which thus becomes an object of the claim.
9. (amended) Agents according to claim 1 ~~to 8~~ for treating tumors, such as colonic cancers and melanomas, or for regenerating and promoting hair growth.

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1116555-10/069896

JC19 Rec'd PCT/PTO 27 FEB 2002

AGENTS FOR TREATING HUMAN DISEASES; ESPECIALLY FOR TREATING TUMORS SUCH AS COLONIC CANCERS AND MELANOMAS OR FOR REGENERATING TISSUE AND PROMOTING HAIR GROWTH

#### Description

The invention relates to agents for treating human diseases based on substances affecting the interaction between  $\beta$ -catenin and LEF-1-/TCF-transcription factors, APC or conductin/axin. Preferably these agents are suitable for treating tumors such as colonic cancers and melanomas or for regenerating tissue and promoting hair growth. Accordingly, fields of application of the invention are pharmaceutical industry and medicine.

$\beta$ -catenin is a cytoplasmic protein which fulfils various functions in the cell. In complex with the cell adhesion molecules of the cadherin family  $\beta$ -catenin establishes the connection with the cytoskeleton (Huelsken J. et al., E-cadherin and APC compete for the interaction with beta-catenin and the cytoskeleton. J-Cell-Biol. 127: 2061-9, 1994). In addition,  $\beta$ -catenin is a component of the Wnt signal transduction which plays an important role in embryonic development. The transcription factor LEF-1 was identified as interaction partner of  $\beta$ -catenin in this signal cascade (Behrens, J. et al., Functional interaction of beta catenin with the transcription factor LEF-1. Nature, 382: 638-42, 1996). The mechanism of signal transduction by  $\beta$ -catenin and LEF-1 has been clarified: It consists of the transport of  $\beta$ -catenin into the cell nucleus mediated by LEF-1. And regulation of the gene expression in the cell nucleus by the LEF-1 induced DNA bending modified in the complex and by the carboxy-terminal transactivation domain of  $\beta$ -catenin. In the mean time, there has been shown that also other members of the LEF-1/TCF family of transcription factors, e.g. TCF-4, are able to

mediate this signal transduction (Korinek, V. et al., Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/-colon carcinoma. Science, 275: 1784-87, 1997).

Stabilizing the cytoplasmic pool of free  $\beta$ -catenin not bound to cadherin is the prerequisite to this signal transduction depending on  $\beta$ -catenin. This pool is negatively regulated by glycogen synthetase kinase 3 $\beta$ , by the tumor suppressor gene product APC and conductin/axin.

There was shown for cancers and melanomas that mutations in the N-terminal area of  $\beta$ -catenin or in the  $\beta$ -catenin binding domain of APC stop this regulation (Morin, P.J. et al., Activation of beta-catenin-Tcf signaling in colonic cancer by mutations in beta-catenin or APC. Science, 275: 1787-90, 1997). Accordingly, the  $\beta$ -catenin pool is stabilized. In melanomas this stabilization results in a LEF-1 mediated translocation of  $\beta$ -catenin into the cell nucleus whereas in colonic cancers this function is primarily fulfilled by TCF-4. The transcriptional activity of the complex in cancer cell lines is detected by activating a reporter gene. In addition, it has been shown that this activity is inhibited in APC-deficient colon carcinoma cell lines after transfection of APC.

APC mutations were identified in the overwhelming majority of colonic cancers whereas not-APC-deficient tumors show mutations in the  $\beta$ -catenin gene. The result of these mutations of APC or  $\beta$ -catenin is an activation of signal transduction by the  $\beta$ -catenin-LEF/TCF complex. This underlines the key role played by  $\beta$ -catenin in the development of tumors. As APC mutations were identified as an early event in the development of colonic tumors the activation of the  $\beta$ -catenin-LEF/TCF complex is certainly a central step in the development of tumors.

It was demonstrated in the mouse, that a deletion of the LEF-1 gene or the expression of  $\beta$ -catenin-mutants, which are stabilized against degradation in the cell, among others lead to troubles of the development of hair follicles. Interestingly is, the expression of stabilised  $\beta$ -catenin leads to increasing the quantity of hair follicle (Gat U. et., 1998. De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. Cell 95:605-14) the inactivation of LEF-1 to trouble to the development of hair follicles, breast glands, and tooth (van Genderen et al., 1994. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1 deficient mice. Genes Dev. 8:2691-703). These results point to, that the complex of LEF/TCF and  $\beta$ -Catenin joins these development processes.

Attempts have been made to utilize the key role played by  $\beta$ -catenin in the development of tumors for the development of therapeutic agents for treating tumors. Nearly at the same time, two patent applications were filed in the USA which, in the mean time, were published as WO papers. In WO 98/41631 (John Hopkins University - B. Vogelstein) the influence on interactions of  $\beta$ -catenin, TCF-4 and the tumor suppressor protein APC aimed at preventing the development of cancer is claimed. There was shown that products of mutated APC genes detected in colorectal tumors are no longer able to regulate the activation of the  $\beta$ -catenin/TCF-4 transcription. Furthermore, colorectal tumors with intact APC genes show activation mutations of  $\beta$ -catenin in the N-terminal area which affects the functioning of the most important phosphorylation sites. Based on this data, the conclusion is drawn that the regulation of  $\beta$ -catenin is critical for the tumor suppressor effect of APC and this regulation may be

evaded by mutations in APC or in  $\beta$ -catenin. The main claim relates to the intron-free DNA molecule coding for TCF-4.

WO 98/42296 (Onyx Pharmaceuticals Inc. - Rubinfeld) relates to compositions and methods of diagnosing and treating illnesses caused by interactions between  $\beta$ -catenin and transcription factors. The main claim relates to the isolated, stabilized  $\beta$ -catenin and its fragments, yet such fragments were not indicated.

Furthermore it was proposed, to find peptids or derived structures, which are from the origin of  $\beta$ -catenin or its interaction compounds which influence the interactions specifically (DE 198 07 390.9 of February 21, 98).

The aim of this invention is providing new agents for the treatment of tumors and aberrant the development of tissues and organs. The invention is based on the task to affect the interaction between  $\beta$ -catenin and LEF/TCF transcription factors, APC and conductin/axin as a prerequisite to the translocation and the activity of the complex in the cell nucleus. This activity has to be specific, i.e. it must not interfere with other interactions of  $\beta$ -catenin, at the same time.

The invention is realised accordingly the claims. An essential basis is - to our surprise - the detection of seperate essential binding sites of these interaction participants in the  $\beta$ -catenin molecule.

The main idea is

- (1) to search for hydrophobic pockets near the essential binding sites of LEF-1/TCF, APC or conductin based on crystal structures and surface calculations for  $\beta$ -catenin. Such a hydrophobic pocket was found near the LEF/TCF binding site and characterized.

- (2) Hydrophobic pockets near the essential binding sites are used for the computer-aided finding of low-molecular substances which may bind in these pockets.
- (3) These substances are tested in an ELISA with recombinant  $\beta$ -catenin and individual interaction participants for the efficiency of inhibition.
- (4) Based on crystallographic analytical data of  $\beta$ -catenin the substances shall be optimized by introducing side groups which are additionally stabilized by bonds with the adjacent amino acid residues of the essential binding sites (e.g. the LEF/TCF binding site: Lys 435, Arg 469, His 470, Lys 508 and Arg 515 or the APC binding site for the 20 amino acid repeat domain: Lys 345, Trp 383 or the 15 amino acid repeat domain or the conductin binding site: Phe 253, His 260, Lys 292).

In following the invention is explained in detail:

In the first realisation of invention got  $\beta$ -catenin- mutants were identified which mark the respective essential binding site for LEF-1/TCF-4, for the 20 amino acid repeats of APC or of conductin (Fig.1). The substitution of single basic amino acids by alanine rest produced this pointmutants. Because of the essential binding sites of this factors are located in separated subregions of  $\beta$ -catenin, the substances near to this binding sites influence always one interaction influence specifically. According to the invention, these substances able treat tumors or regenerate tissues.

In a second step, based on the X-ray crystallographic analytical data of the armadillo domain of  $\beta$ -catenin the surfaces in this region are calculated. Using various computer programs such as e.g. Grasp or Ludi a hydrophobic pocket near the binding site for LEF-1/TCF-4 was identified (flanked by the amino acids Val 358, Met 363, Ala 391, Ala 392, Thr 393, Lys 394, Gln 395, Met 398,



In a third step low-molecular substances from molecule data bases were computer-aided fitted into this pocket and selected owing to the number of the stabilizing intractions with  $\beta$ -catenin. These substances are mentioned hereinafter. The basic structure of these low-molecular compounds shall be modified after experimentally checking their inhibition function (e.g. in an ELISA) to optimize their function. This is guided by the idea that the modification of the substances, e.g. by adding acid groups, allows additional interactions with the basic residues of the amino acids Lys 435, Arg 469, His 470 or Lys 508 of  $\beta$ -catenin. As these residues mark the essential binding site of the LEF/TCF factors these interactions should intensify the efficiency of the substances.

The adoption of this strategy to all essential binding sites of  $\beta$ -catenin allows to generate new pharmaca, which by specifically affecting the respective interaction of  $\beta$ -catenin, e.g. with the transcription factors LEF/TCF, the tumor suppressor gene product APC or conductin, inhibit the development of tumors or are used for regenerating skin and promoting growth of hair. These



possibilities of affecting arise from the findings published which relate to the function of the respective interaction between  $\beta$ -catenin and its binding participants in these developmental processes.

In particular the following investigations were carried out:

1. Identification of separate, essential binding sites of  $\beta$ -catenin for the interaction with LEF-1/TCF-4 with the 20 and 15 amino acid repeats of APC or conductin/axin.

Based on the X-ray crystallographic analysis of the armadillo domain of  $\beta$ -catenin (Huber et al., 1997) selected basic and aromatic amino acid residues were substituted by alanine residues and the point mutants were analysed for their interaction with the binding participants of  $\beta$ -catenin in a yeast-2-hybrid system (Fig. 1). The mutations blocking specific interactions form clusters in separate subregions of the domain. They mark the essential points of contact in the specific binding sites of  $\beta$ -catenin for its interaction with LEF/TCF (Lys 435, Arg 469, His 470, Lys 508, Arg 515), with the 20 amino acid repeats of APC (Trp 383, Lys 345), with the 15 amino acid repeats of APC (Arg 386), or with conductin (Phe 253, His 260, Lys 292). The extended interaction sites are represented in Fig. 1.

2. Analyses of the molecule surface of  $\beta$ -catenin in the area of the essential binding site for LEF/TCF

Proceeding from the X-ray crystallographic analytical data of  $\beta$ -catenin the molecule surface was calculated by means of the programs Grasp and Ludi in the area of the binding site. Thus, it was possible to identify a hydrophobic pocket (Fig. 2) flanked by the following amino acids: Val 358, Met

363, Ala 391, Ala 392, Thr 393, Lys 394, Gln 395, Met 398, Leu 401, Leu 402, Ile 423, Asn 426, Leu 427, Thr 428, Cys 429, Asn 430, Asn 431, Asn 434, Met 437, Val 438. This pocket is localised in the closest vicinity of the essential binding site for LEF/TCF. It is of great importance for the computer-aided screening of substance libraries for selecting molecules binding in this pocket. The idea to select primarily hydrophobic interactions in the pocket as starting point provides the energetic advantage that the substances need not compete with the hydrate shell of the loaded amino acid residues of the surface. Thus, after their purposeful modification these substances are potent therapeutic agents for treating tumors as the interaction of LEF/TCF with  $\beta$ -catenin is an early event in the development of tumors. They are experimentally tested for their ability to inhibit the oncogenic interaction (ELISA) and are modified. The molecular modelling of the substances in the pocket is based on the idea to stabilize the interactions proceeding in the proper pocket and to establish additional contacts to the essential amino acid residues of the LEF/TCF binding site. By means of this strategy the effect of these substances is to be optimized.

### 3. Identification of substances binding in the hydrophobic pocket

Substance libraries were screened computer-aided and the molecules were selected according to their stabilizing interactions in the hydrophobic pocket. The selected substances were checked experimentally to inhibition of the complex formation of  $\beta$ -catenin with LEF-1 in an ELISA. The basic structures of the molecules detected form various molecule classes which are represented in the following:

**Molecule class I:**

- A:** Cephalosporines of similiar structure as the substance "cefamandole" with an inhibition of complex formation for  $\beta$ -catenin and LEF/TCF ( $IC_{50} = 25-100 \mu M$ ). The lead structure consists of an aromatic ring (in the case of cefamandole at carbon atom 17, compare "positive list"), an organic component resembling the structure of the ala-ala dipeptide and of a  $\beta$ -lactam and thiazole ring. By means of the armatic ring the substance was fit into the hydrophobic pocket described. Related cephalosporines without this aromatic ring do not show an inhibitory effect. Additionally, stabilizing hydrogen bridges between a carbonyl group (carbon atom 17) of the substance with the Ser 389 of  $\beta$ -catenin and a carboxyl group (carbon atom 8, at the thiazole ring) and the Lys 394 of  $\beta$ -catenin were calculated. Further representatives of this molecule class with an experimentally confirmed inhibition of complex formation are the cephalosporines cefsulodine and cefadroxil ( $IC_{50} = 50-100 \mu M$ ). Basically claim is raised for all cephalosporines with the same lead structure as that of cefamandole to provide the guiding structure for the development of a therapeutic agent or unmodified as a therapeutic agent for combating tumors and cancer.
- B.** As to its structure substance AC-(6-O-stearoyl) muramyl-ala-D-isoglutamine forms part of another subclass of inhibitors of complex formation ( $IC_{50} = 100 \mu M$ ).
- C:** As to its structure substance 3,6 dihydroxybenzonobornane forms part of another subclass of inhibitors of complex formation ( $IC_{50} = 100 \mu M$ ).



was quantified by means of the  $\beta$ -galactosidase reporter activity in the yeast 2-hybrid system (report:"Matchmaker", Clontech) (Fig. 1).

2. Identification of a hydrophobic pocket in  $\beta$ -catenin in the vicinity of the essential binding site for LEF-1. To calculate the molecule surface of  $\beta$ -catenin various computer programs such as GRASP, SPOCK and LUDI of MSI were used.

3. Identification of substances binding in the hydrophobic pocket at the essential binding site for LEF-1  
Various substance libraries, e.g. the available chemicals data base, ACD of MSI and the programs LUDI, SPOCK and GRASP were used for searching substances binding in the hydrophobic pocket.

4. ELISA for testing substances for their inhibition of the complex formation of  $\beta$ -catenin and LEF-1.  
Selected substances were tested for their inhibition of the interaction between LEF-1 and  $\beta$ -catenin in producing at first proteins in bacteria recombinant with N-terminale histidin-sequences and dry-cleaning with nickel-chromatography (Behrens et al.1996).About 50 ng LEF-1 was adsorbed on the wells of ELISA-plates for 60 minutes at roomtemperature in PBS/BSA (0,1 mg BSA/ml).Following the wells were covered with 2,5% skimmed milk/0,5%BSA for 2 hours at 8°C.All the further steps followed at room temperature in PBS/BSA (10 $\mu$ g BSA/ ml).

After washing the wells with PBS the substances dissolved in ad 10 % DMSO final and the indicated final concentrations of the substances were added.

The incubation with 50-100 ng  $\beta$ -catenin was realized for 15 minutes in PBS/BSA (0,5mg BSA/ml). The complex formation of LEF-1 and  $\beta$ -catenin was detected with the antibody PA2 against carboxy-

terminus of  $\beta$ -catenin (Hülsken et al 1994). PA2 was added for 15 minutes in a titre dilution of 1:10000 in 1% BSA/PBS. After washing the wells with PBS the quantification of the complex-formation followed by peroxidase conjugated antibodies (1:2000 in 1% BSA/PBS, Dianova) and by photometrically measuring of the turnover of o-phenyldiamin as substrate at 450 nm (Ultramark ELISA reader, BioRad). The substances were used in concentrations between 1  $\mu$ M and 10 mM. To check the specific inhibition of the complex formation of LEF-1 and  $\beta$ -catenin,  $\beta$ -catenin was absorbed in the wells and detected after incubation with the substances. Substances binding in the hydrophobic pocket of  $\beta$ -catenin without impeding the complex formation with LEF-1 were identified owing to their competition for this binding site with substances showing inhibitory effects. As example, cefamandole was used as a substance showing an inhibitory effect in its semimaximally efficient concentration ( $IC_{50} = 100 \mu$ M) together with another substances of the same concentration (100  $\mu$ M) in each binding reaction in an ELISA. Substances, which bond at the same place as cefamandole, but used don't inhibit alone the complex formation, don't influence the cefamandole binding.

#### Legends for the Figures and Tables:

Fig. 1: Identification of the essential binding sites of  $\beta$ -catenin for LEF-1, the 20 amino acid and 15 amino acid repeats of APC or conductin

(A-D) Interaction of  $\beta$ -catenin mutants with their binding partners in the yeast-2-hybrid system. The amino acid residues substituted by alanine in the  $\beta$ -catenin mutants and their position in the arm repeats 3-8 are given hereinafter. The interaction was quantified by determining the  $\beta$ -galactosidase reporter activity and is indicated compared with the interaction



with wild type  $\beta$ -catenin. (E) Description separately essential binding sites in the Armadillo- domain of  $\beta$ -catenin (RasMol).

Fig. 2: Characterization of a hydrophobic pocket adjacent to the essential binding sites of  $\beta$ -catenin for LEF-1/TCF

(A) View of the hydrophobic pocket at the molecule surface of  $\beta$ -catenin (RasMol). The pocket is flanked by amino acids marked in orange or yellow colours. The amino acid residues of the essential binding site for LEF/TCF are marked in blue colour. The respective amino acids have been marked. (B) Side view of the hydrophobic pocket.

Fig. 3: Substances binding in the hydrophobic pocket of  $\beta$ -catenin

(A) Representation of the surface of the hydrophobic pocket region (Grasp). The amino acid residues of the essential binding site for LEF/TCF are marked in blue colour (for mutations blocking the interaction between  $\beta$ -catenin and LEF/TCF: Lys435, Arg 469 and His 470). (B) In the  $\beta$ -catenin molecule one of the low-molecular substances binding in the pocket is represented.

Fig. 4: Cefamandole as a representative of molecule class I inhibits the complex formation of LEF-1 and  $\beta$ -catenin in an ELISA.

Rising concentrations of cefamandole (15-250  $\mu$ M) result in a reduction of the complex formation of LEF-1 and  $\beta$ -catenin protein prepared recombinantly and purified in an ELISA (IC<sub>50</sub>=25  $\mu$ M).

Table 1: Substances potentially binding in the hydrophobic pocket ("drug list"), calculated by computer calculations ( for example LUDI/MSI)



Table 2: "Positive list" of substances inhibiting the complex formation of  $\beta$ -catenin and LEF-1 in ELISA or influence the effect from cefamandole.



- the  $\beta$ -catenin-mutants Lys 345, Trp 383, Arg 386, which mark the essential binding site for APC were identified,
  - the molecule surface is calculated with the aid of the programs Grasp, Ludi and similar programs,
  - a hydrophobic pocket (flanked by the amino acids Val 358, Met 363, Ala 391, Ala 392, Thr 393, Lys 394, Gln 395, Met 398, Leu 401, Leu 402, Ile 423, Asn 426, Leu 427, Thr 428, Cys 429, Asn 430, Asn 431, Asn 434, Met 437, Val 438) was identified in the vicinity of the LEF-1/TCF binding site,
  - the compounds according to the annexed "drug list" and positive list" which thus become a subject of the claim are fitted into the identified hydrophobic pocket, subsequently experimentally checked with ELISA for their inhibition of the complex formation of  $\beta$ -catenin and its binding partners and optimized by chemical modification.
5. Substances with a lead structure essential for binding in the hydrophobic pocket and inhibition of the formation of complexes with  $\beta$ -catenin marked by cephalosporines of the cefamandole type (molecule class IA), with the lead structure essential for binding in the hydrophobic pocket and inhibition of the formation of complexes with  $\beta$ -catenin consisting of an aromatic ring, an organic component similar to the structure of the ala-ala dipeptide and a  $\beta$ -lactam and thiazole ring, which are preferably cephalosporines of the cefamandole type such as e.g. cefsulodine, cefadroxil or cefamandole nafates.
  6. Substances for the inhibition of the complex formation of  $\beta$ -catenin with LEF/TCF marked by a structure corresponding to AC-(6-0-stearoyl)-muramyl-ala-D-isoglutamine (molecule class IB).

7. Substances for the inhibition of the complex formation of  $\beta$ -catenin with LEF/TCF marked by a structure corresponding to that of 3,6-dihydroxybenzonobornane (molecule class IC).
8. Substances binding in the hydrophobic pocket without inhibiting the formation of complexes, however affecting binding of cefamandole to  $\beta$ -catenin as guiding structures for developing potent inhibitors, preferably substances of the «positive list» table which thus becomes an object of the claim.
9. Agents according to claim 1 to 8 for treating tumors, such as colonic cancers and melanomas, or for regenerating and promoting hair growth.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES  
PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum  
Internationales Büro



(43) Internationales Veröffentlichungsdatum  
22. März 2001 (22.03.2001)

PCT

(10) Internationale Veröffentlichungsnummer  
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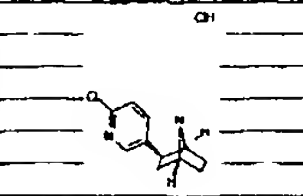
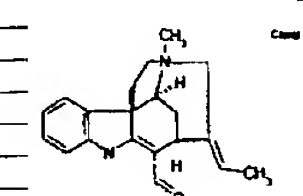
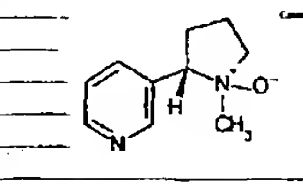
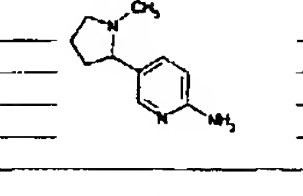
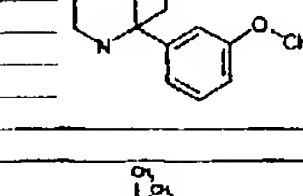
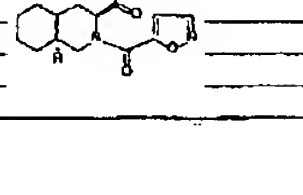
- (51) Internationale Patentklassifikation<sup>7</sup>: C07K 14/47, (71) Anmelder (für alle Bestimmungsstaaten mit Ausnahme von  
G01N 33/50, 33/574 US): MAX-DELBRÜCK-CENTRUM FÜR MOLEKU-  
LARE MEDIZIN [DE/DE]; Robert-Rössle-Strasse 10,  
13125 Berlin (DE)
- (21) Internationales Aktenzeichen: PCT/DE00/03104
- (22) Internationales Anmeldedatum: 7. September 2000 (07.09.2000) (72) Erfinder; und  
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VON KRIES, Jens-Peter [DE/DE]; Meraner Strasse 49 b,  
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- (25) Einreichungssprache: Deutsch
- (26) Veröffentlichungssprache: Deutsch
- (30) Angaben zur Priorität: 199 44 404.8 16. September 1999 (16.09.1999) DE (74) Anwalt: BAUMBACH, Fritz; Robert-Rössle-Strasse 10,  
13125 Berlin (DE).

[Fortsetzung auf der nächsten Seite]

(54) Title: AGENTS FOR TREATING HUMAN DISEASES, ESPECIALLY FOR TREATING TUMORS SUCH AS COLONIC  
CANCERS AND MELANOMAS OR FOR REGENERATING TISSUE AND PROMOTING HAIR GROWTH

(54) Bezeichnung: MITTEL ZUR THERAPIE VON MENSCHLICHEN ERKRANKUNGEN, INSBESONDERE FÜR DIE THE-  
RAPIE VON TUMOREN WIE KOLONKARZINOMEN UND MELANOMEN ODER ZUR GEWEBEREGENERATION UND  
FÖRDERUNG DES HAARWUCHSES

drugs-liste 2000

	(+)-EPIBATIDINE HYDROCHLORIDE	MFCD00467208
	FLUOROURACINE CHLORIDE	MFCD00467712
	(1'S,2'S)-NICOTINE 1'-OXIDE	MFCD00869528
	SPECS SPECS CIF7952	MFCD01114864
	2-ETHYL-2-(3- METHOXYPHENYL)PYRROLIDINE	MFCD01314146
	N-(TERT-BUTYL)-2-(ISOXAZOL-5- YLCARBONYL)DECAHYDROISOQUINOLINE-3- CARBOXAMIDE	MFCD01314517

(57) Abstract: The invention relates to agents for treating human diseases which are based on substances that specifically influence the binding of  $\beta$ -catenin on LEF-1/TCF- transcription factors, APC or conductin/axin. The invention particularly relates to the identification and use of hydrophobic pockets on the molecular surface in the proximity of the essential binding points for the binding partners of  $\beta$ -catenin with the aim of optimizing these substances. The invention also relates to the use of the substances, preferably for treating tumors such as colonic cancers and melanomas or for regenerating tissue and promoting hair growth.

(57) Zusammenfassung: Die Erfindung betrifft Mittel zur Therapie von menschlichen Erkrankungen auf der Basis von Substanzen, die spezifisch die Bindung von  $\beta$ -Catenin an LEF-1/TCF-Trankriptionsfaktoren, APC oder Conductin/Axin beeinflussen. Sie betrifft insbesondere die Identifikation und Nutzung von hydrophoben Taschen auf der Moleküloberfläche in der Nähe der essentiellen Bindungsstellen für die Bindungspartner von  $\beta$ -Catenin mit dem Ziel, diese Substanzen zu optimieren. Sie betrifft ferner die Anwendung der Substanzen, bevorzugt für die Therapie von Tumoren wie Kolonkarzinomen und Melanomen oder zur Geweberegeneration und Förderung des Haarwuchses.

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Fig. 1A

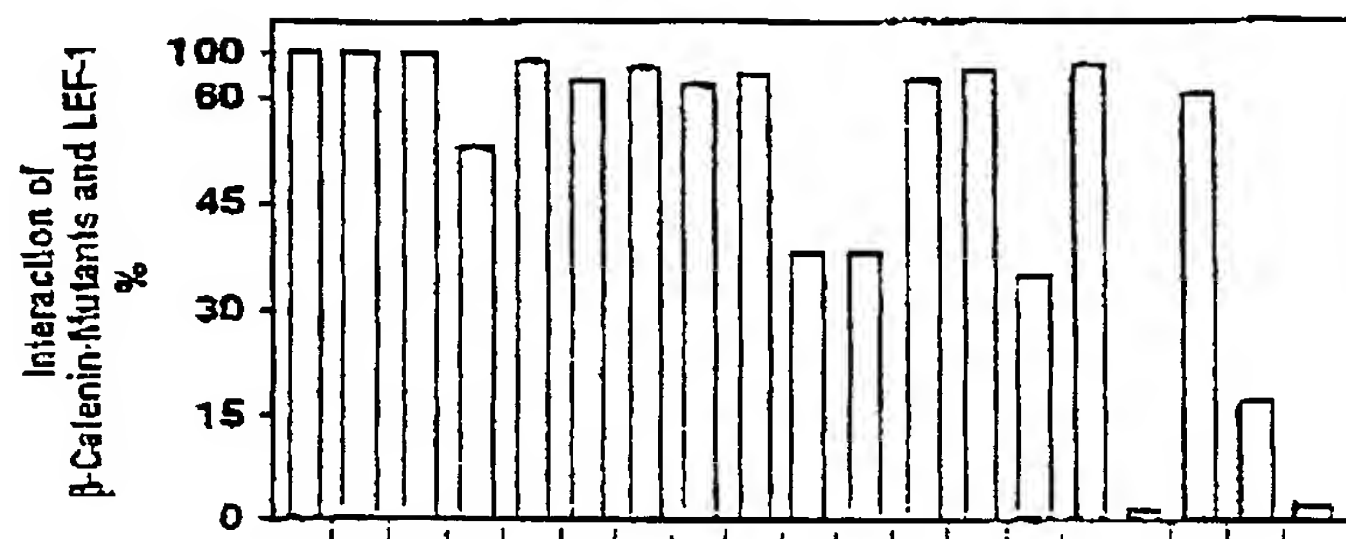


Fig. 1B

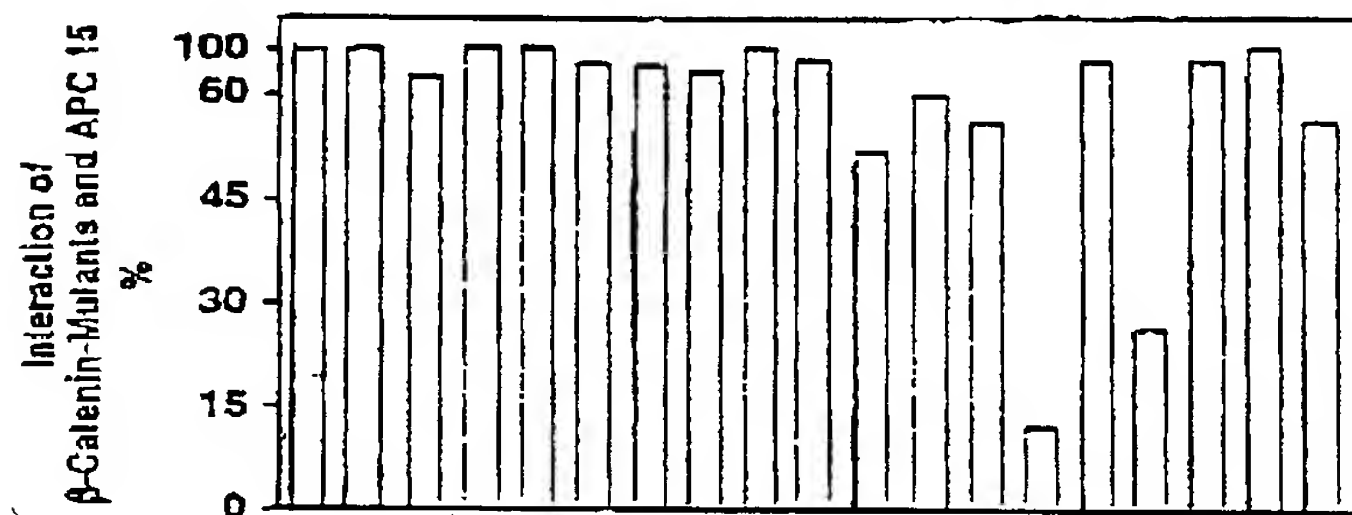


Fig. 1C

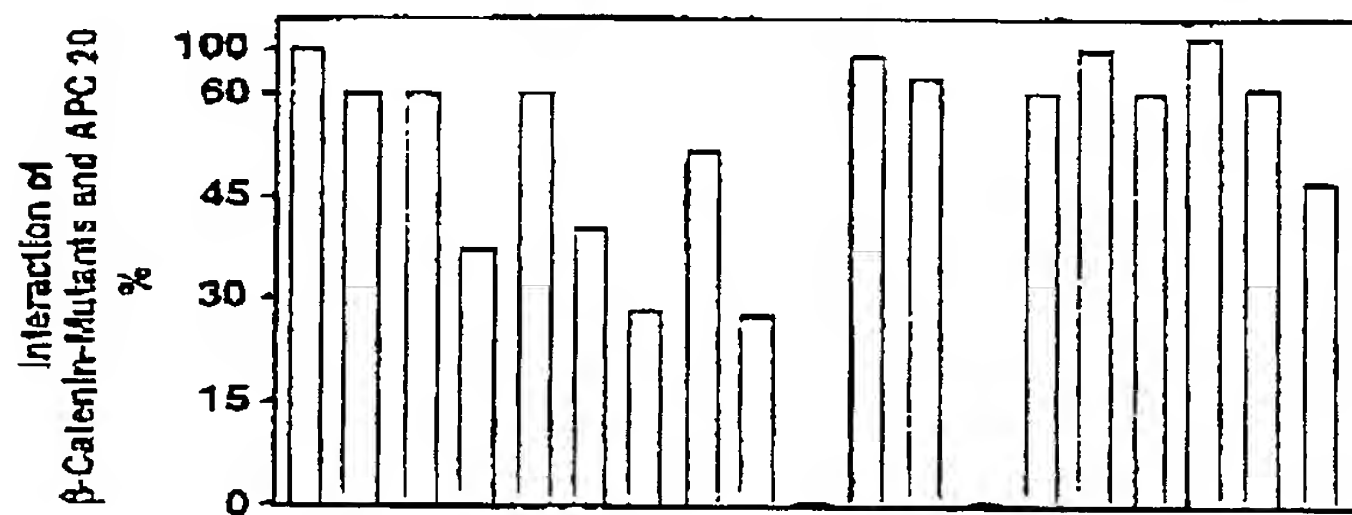
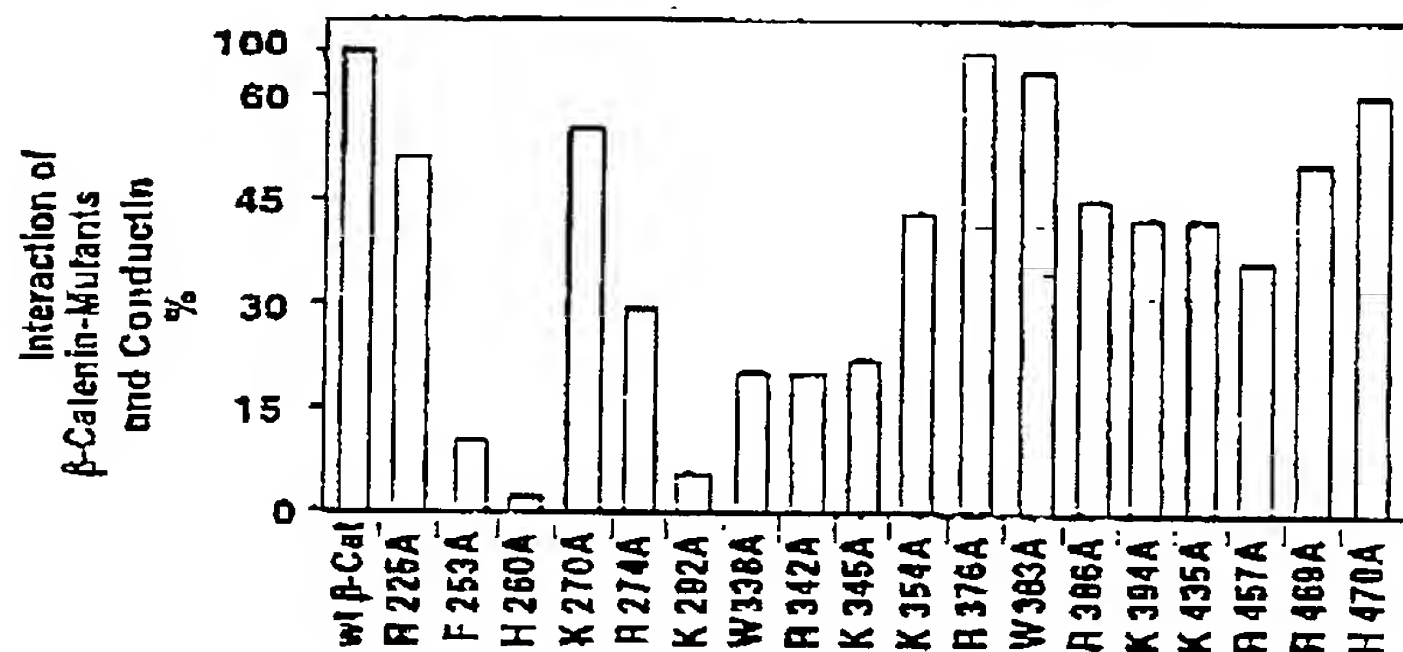


Fig. 1D

arm  
repeats

3	4	5	6	7	8
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Mutants of β-Catenin

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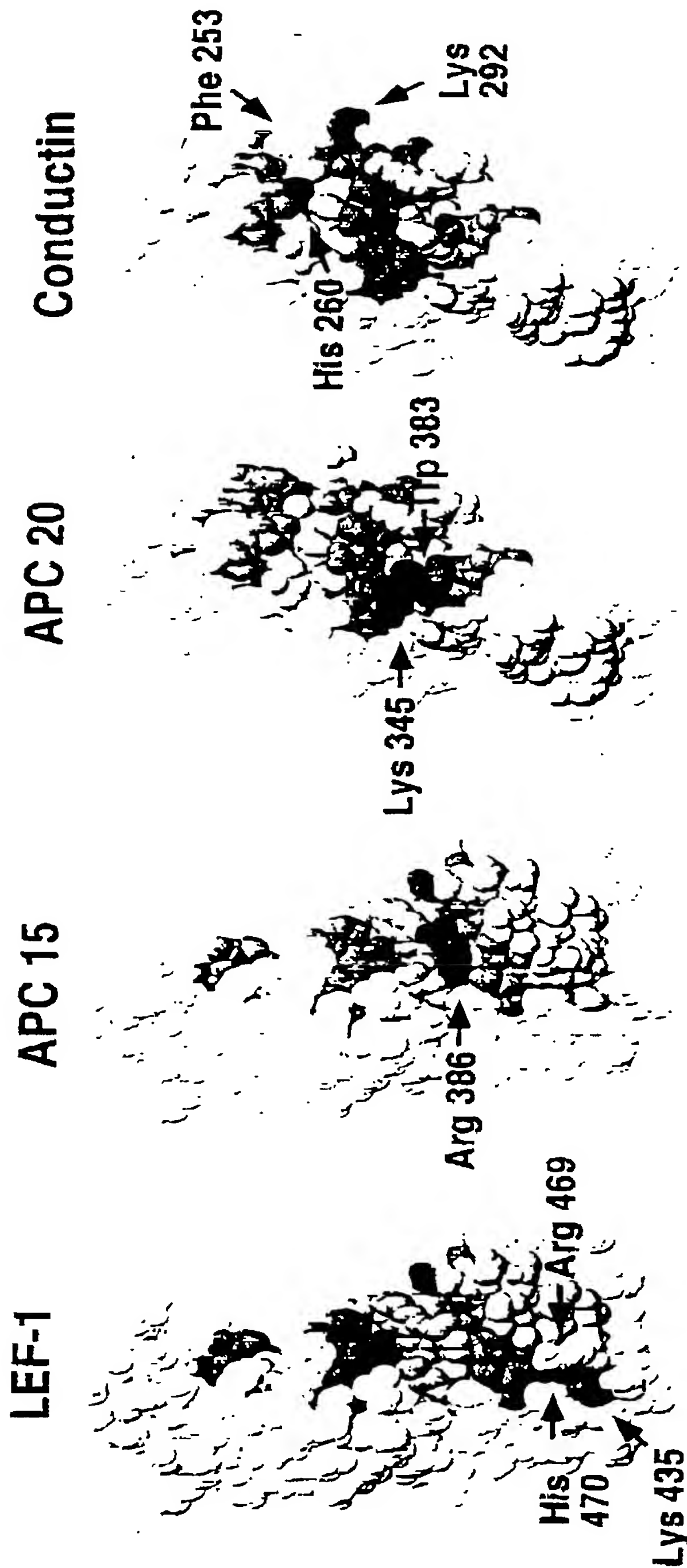


Fig. 1E



Fig. 2A

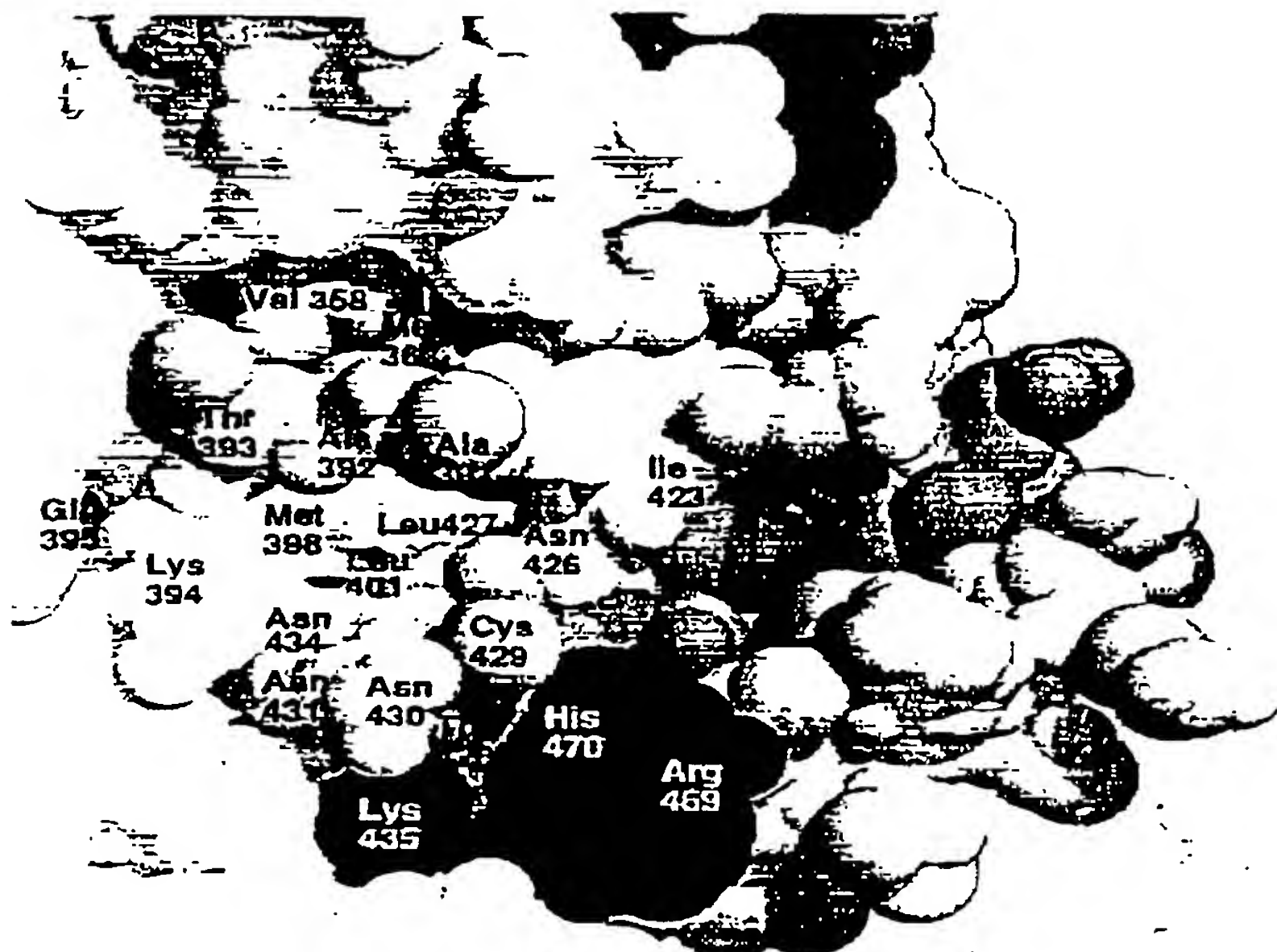
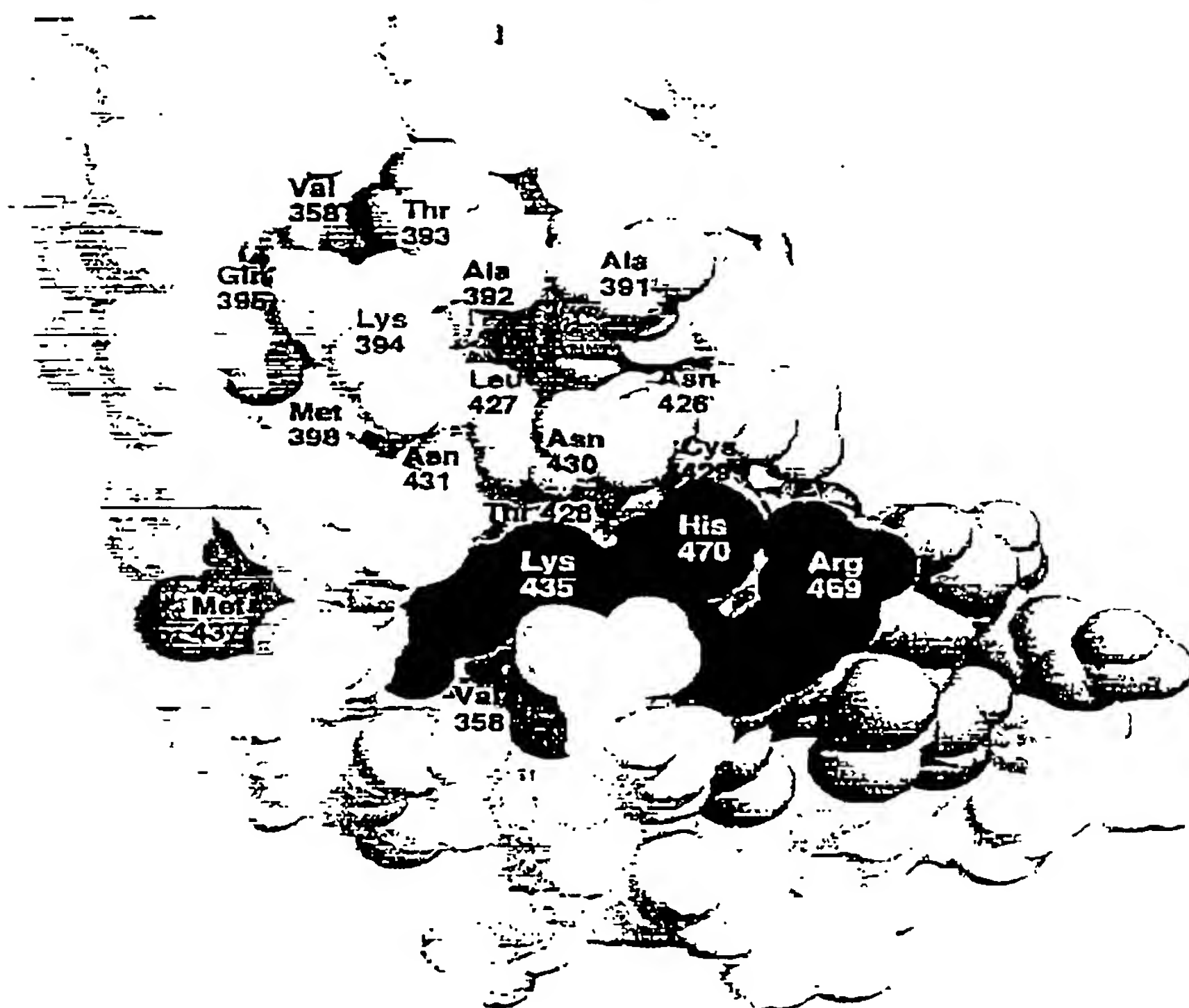


Fig. 2B



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Fig. 3B



Fig. 3A



ERSATZBLATT (REGEL 26)

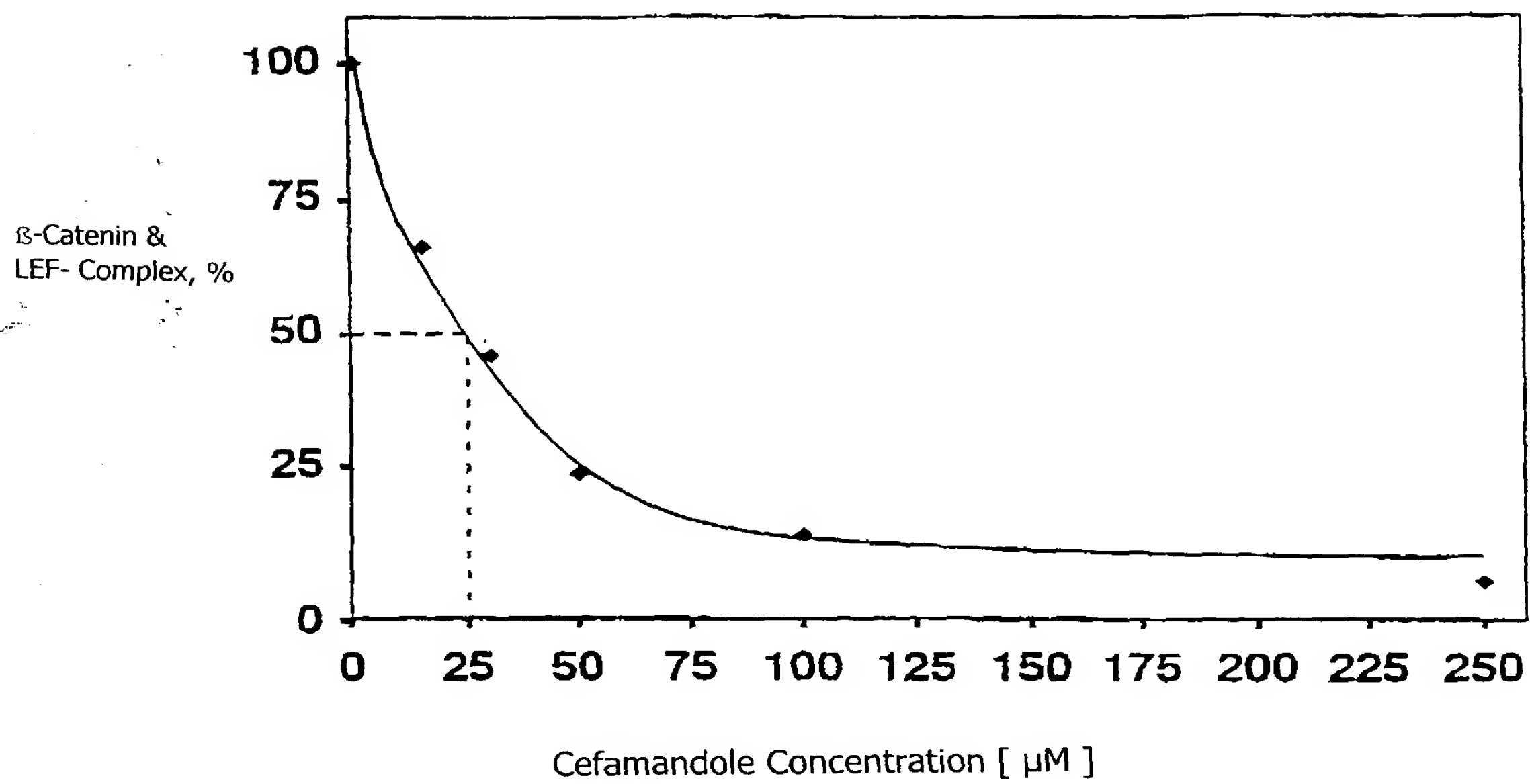
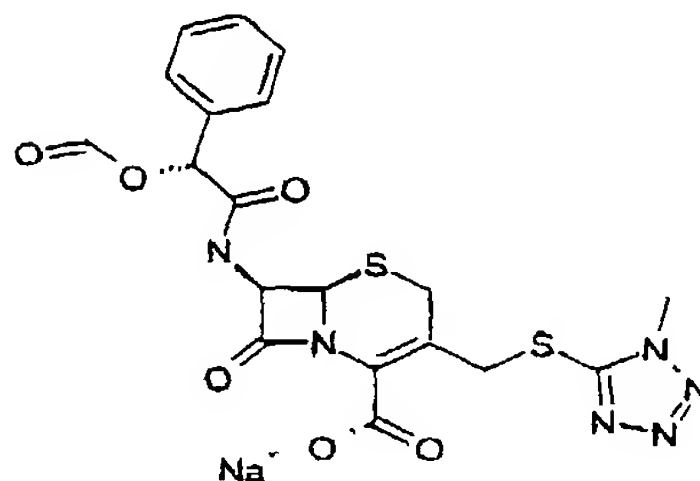


Fig. 4

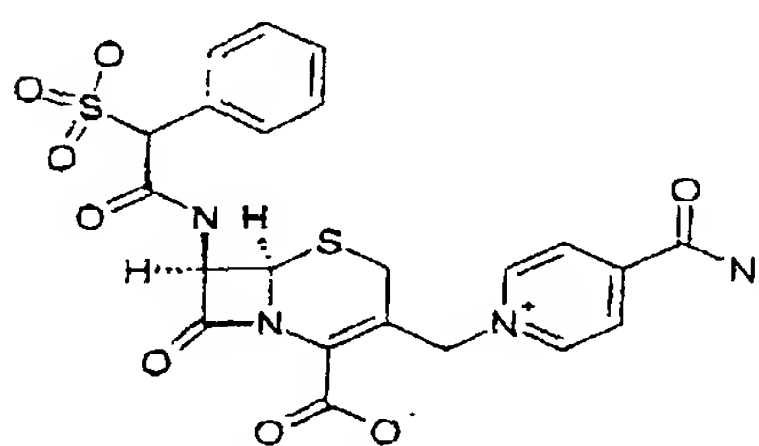
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### Positive List

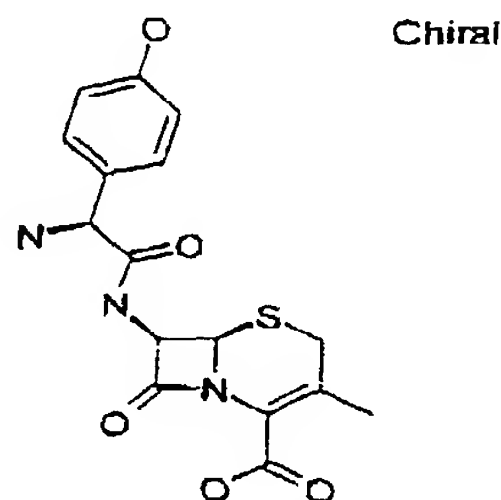
## Cefamandole



## Cefamandole-Nafate

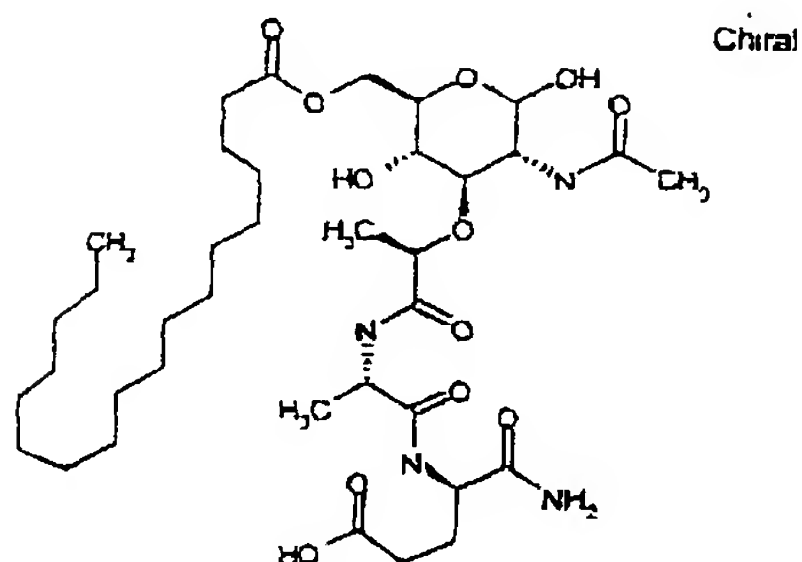


## Cefsulodin



## Cefadroxil

### Molecule-Class 1B



**AC-(6-O-STEAROYL)-MURAMYL-ALA-D-ISOGLUTAMINE**

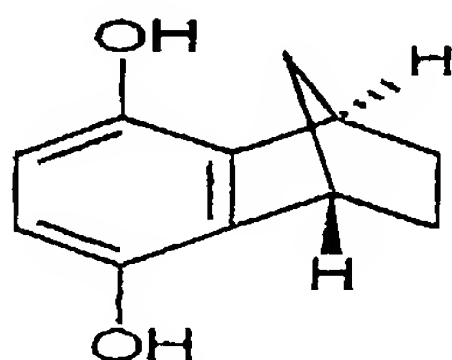
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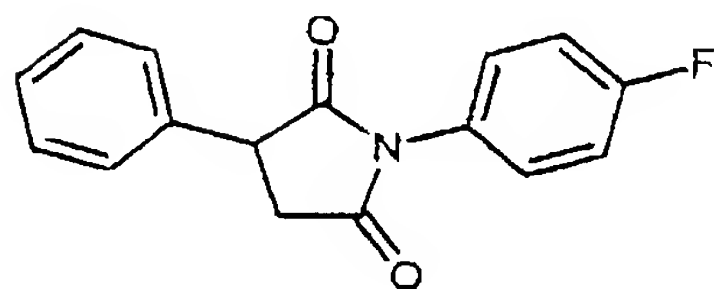
Positive List

### Molecule-Class 1C

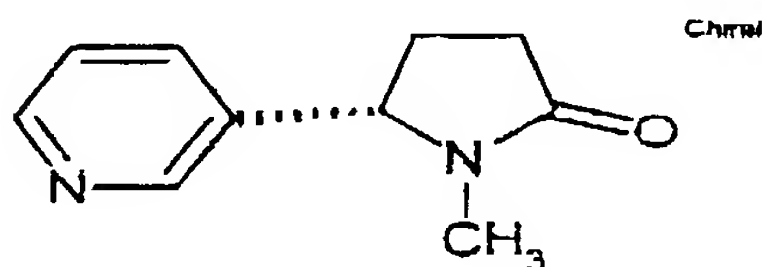


3,6-DIHYDROXYBENZONORBORNANE

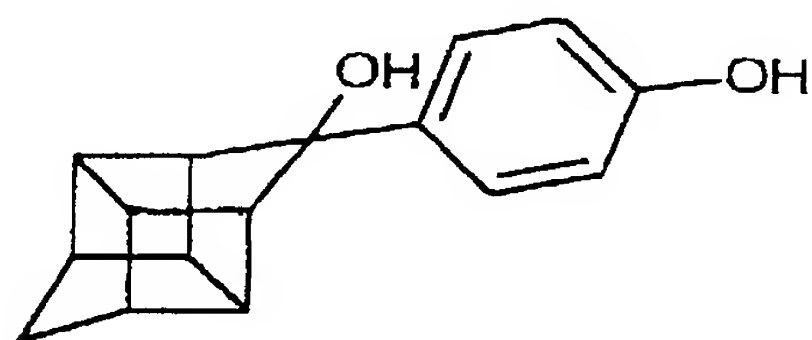
### Molecule with Modulation Effect for molecule class 1



1-(4-FLUOROPHENYL)-3-PHENYLPYRROLIDINE-2,5-DIONE



(-)-COTININE



4-(2-HYDROXYOCTAHYDRO-1,3,4-METHENO-2H-CYCLOBUTA(CD)PENTALEN-2-YL)PHENOL

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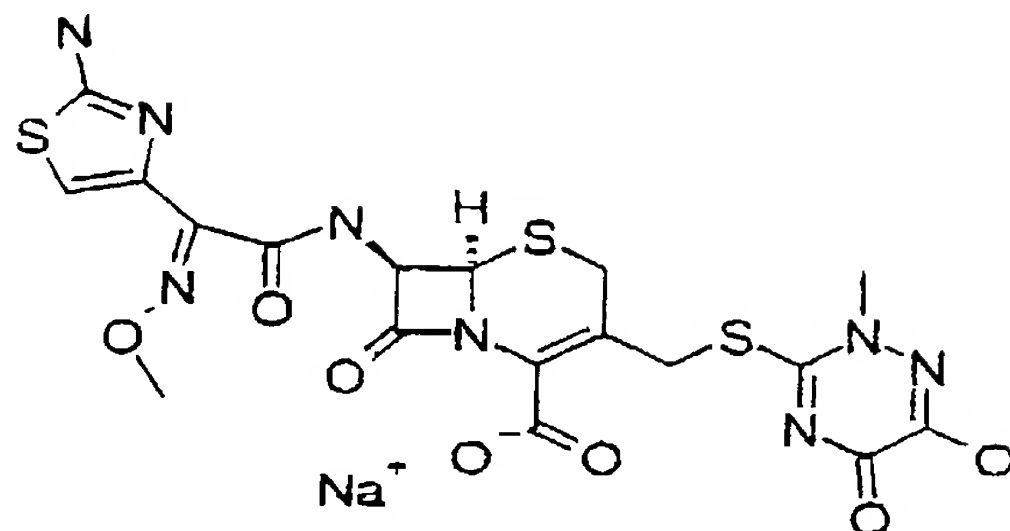
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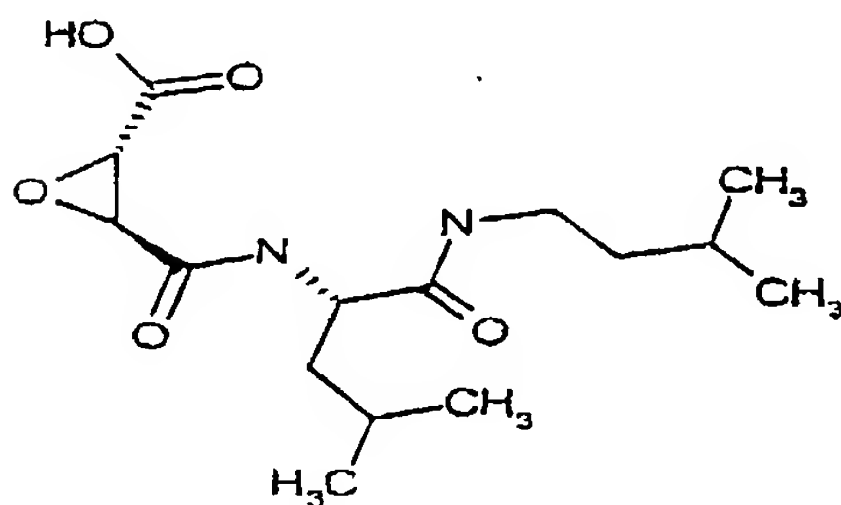
Positive List

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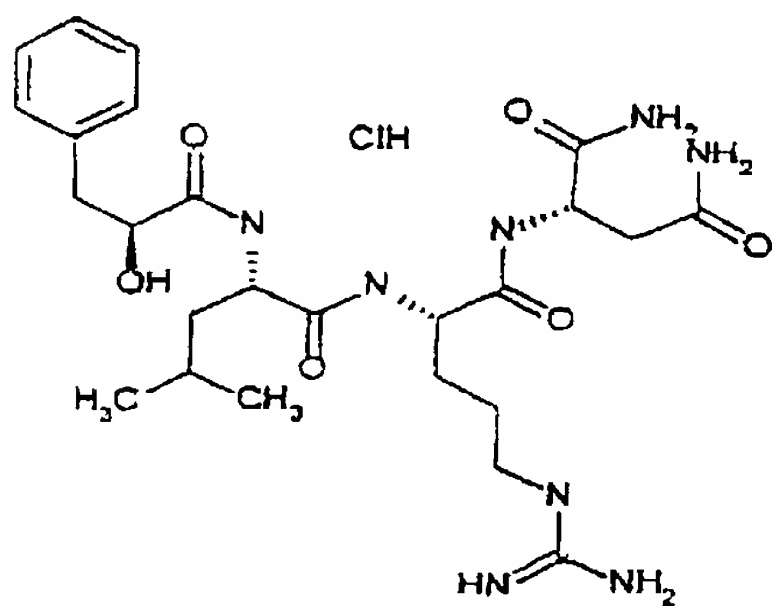
Cefatriaxone

Chiral



L-TRANS-EPOXYSUCCINYL-LEU-3-METHYLBUTYLAMIDE

Chiral



ANTHO-RNAMIDE

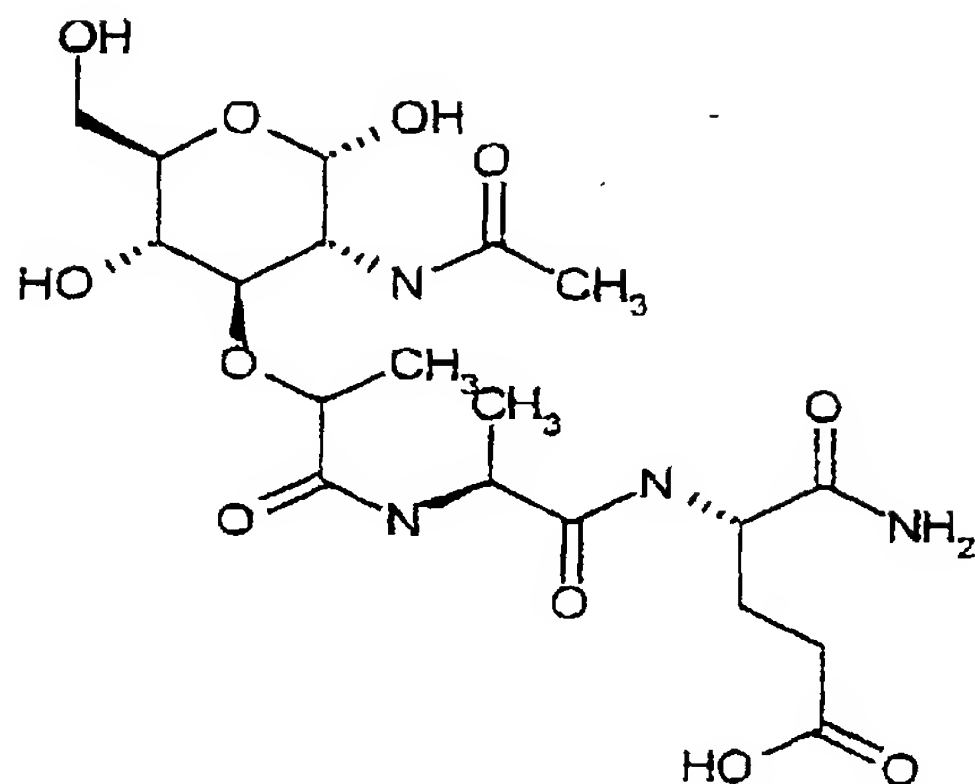
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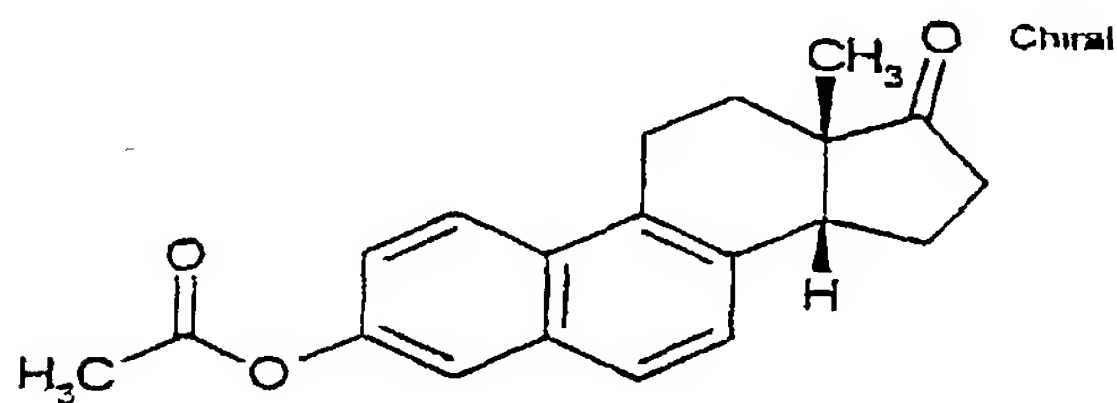
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Positive List

Chiral



N-ACETYL-MURAMYL-ALA-ISOGLN-OH



1,3,5(10),6,8(14)-ESTRAPENTAEN-3-OL-17-ONE ACETATE

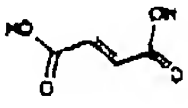
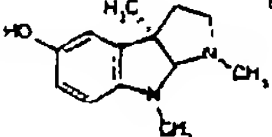
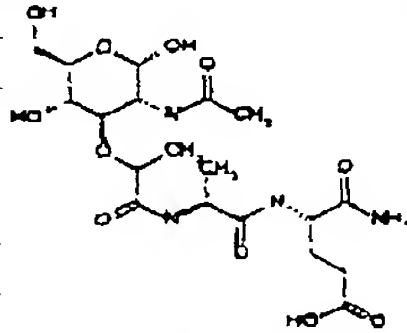
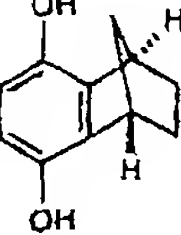
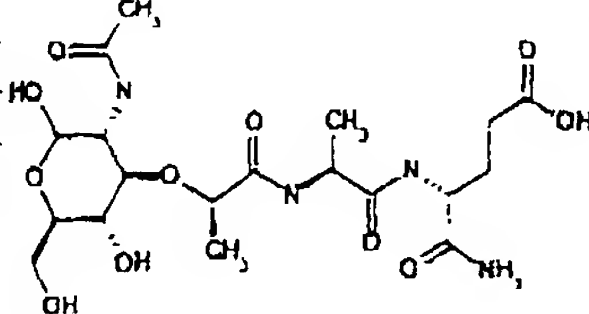
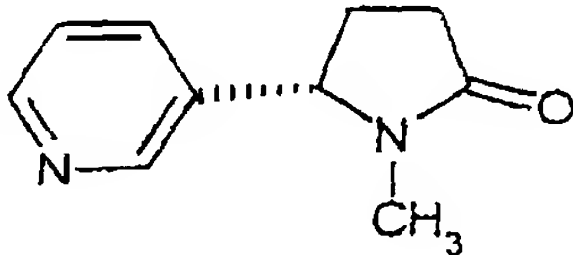
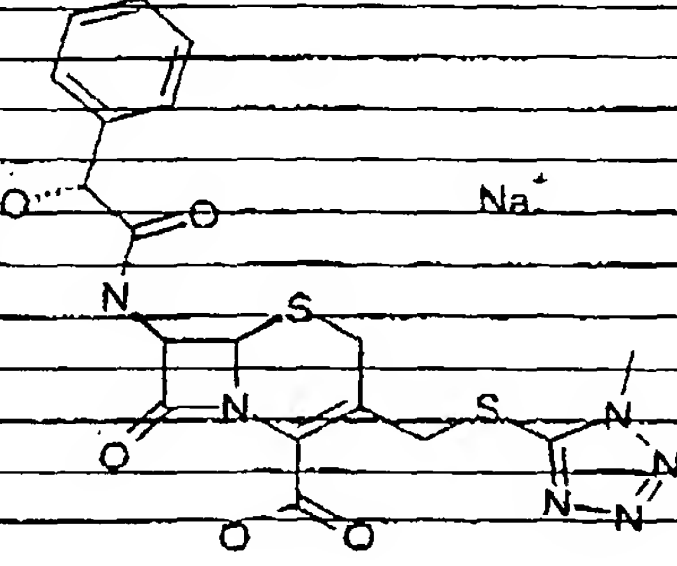


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drugs-list 2000

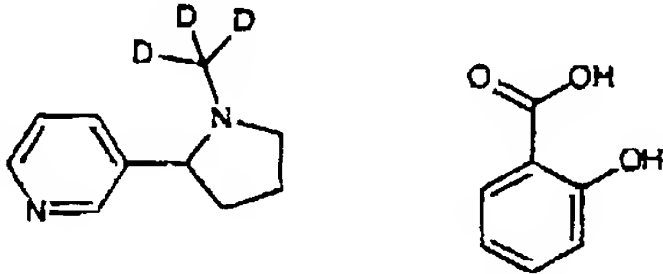
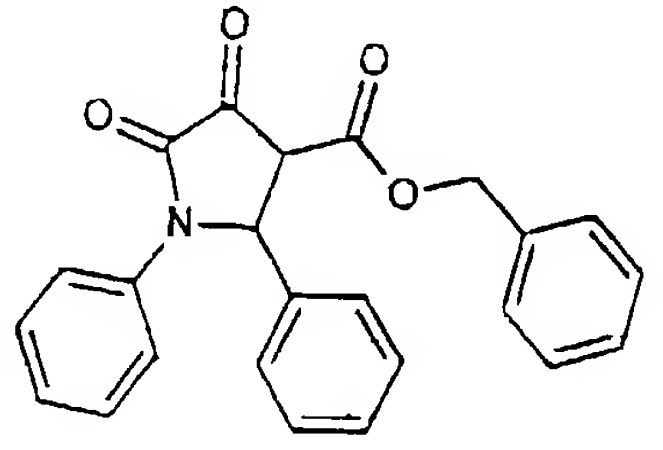
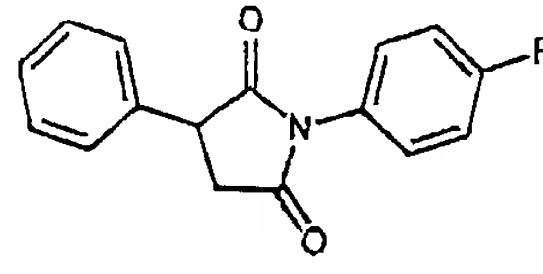
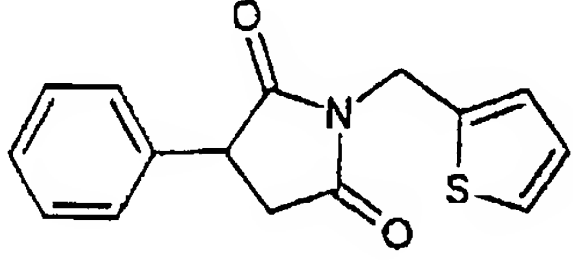
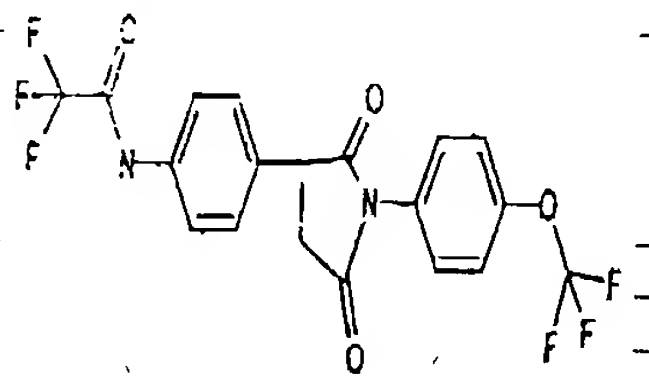
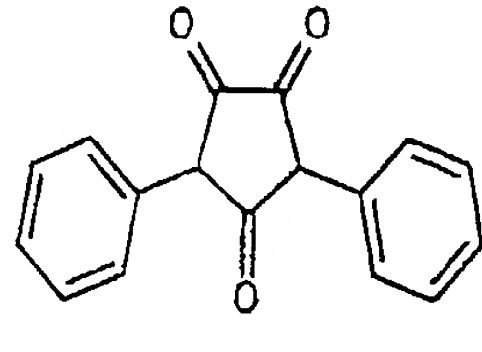
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	3,6-DIHYDROXYBENZONORBORNANE	MFCD00077441
	N-ACETYL-MURAMYL-ALA-D-ISOGLN-OH	MFCD00077638
	(-)-COTININE	MFCD00077696
	CEFAMANDOLE SODIUM SALT	MFCD00082385

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	BENZYL 1,2-DIPHENYL-4-HYDROXY-5-OXO-3-PYRROLINE-3-CARBOXYLATE	MFCD00088051
	1-(4-FLUOROPHENYL)-3-PHENYLPYRROLIDINE-2,5-DIONE	MFCD00097831
	3-PHENYL-1-(2-THIENYLMETHYL)PYRROLIDINE-2,5-DIONE	MFCD00097832
	N1-(4-[2,5-DIOXO-1-(4-(TRIFLUOROMETHOXY)PHENYL]TETRAHYDR O-1H-PYRROL-3-YL]PHENYL)-2,2,2-TRIFLUOROACETAMIDE	MFCD00100474
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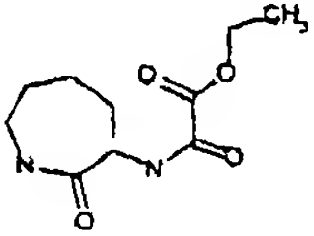
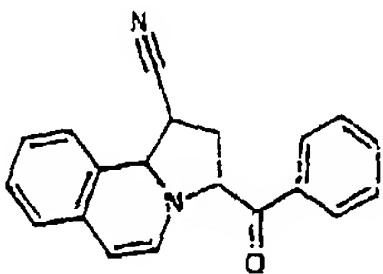
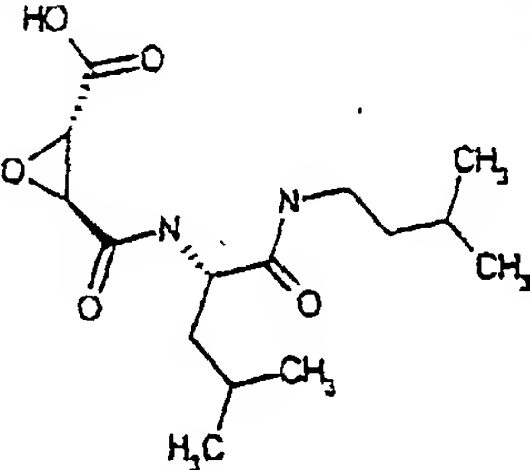
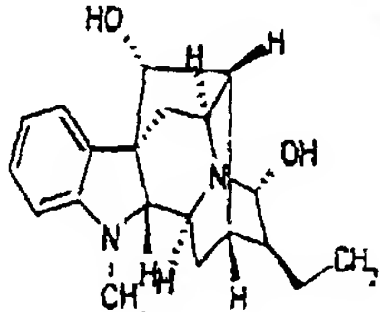
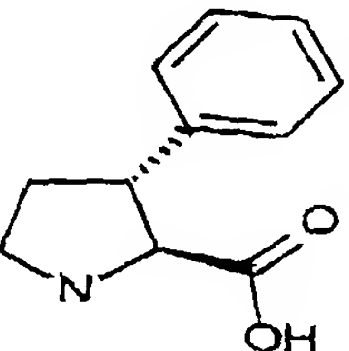
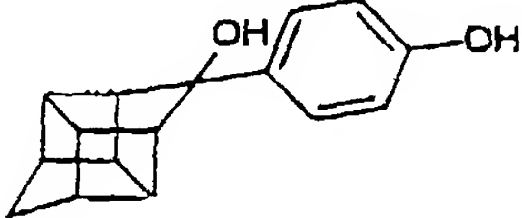
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	ETHYL 2-OXO-2-[(2-OXOAZEPAN-3- YL)AMINO]ACETATE	MFCD00103142
	3-BENZOYL-1,2,3,10B-TETRAHYDRO- PYRROLO(2,1-A)ISOQUINOLINE-1- CARBONITRILE	MFCD00123443
	Chiral L-TRANS-EPOXYSUCCINYL-LEU-3- METHYLBUTYLAMIDE	MFCD00132882
	Chiral AJMALINE	MFCD00135652
	Chiral (2S,3R)-3-PHENYLPYRROLIDINE-2- CARBOXYLIC ACID	MFCD00142984
	4-(2-HYDROXYOCTAHYDRO-1,3,4-METHENO- 2H-CYCLOBUTA(CD)PENTALEN-2- YL)PHENOL	MFCD00155174

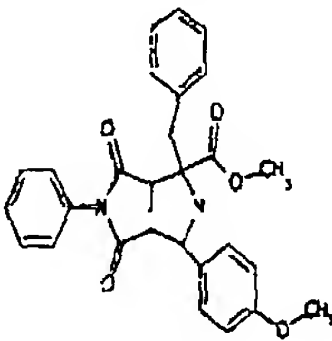
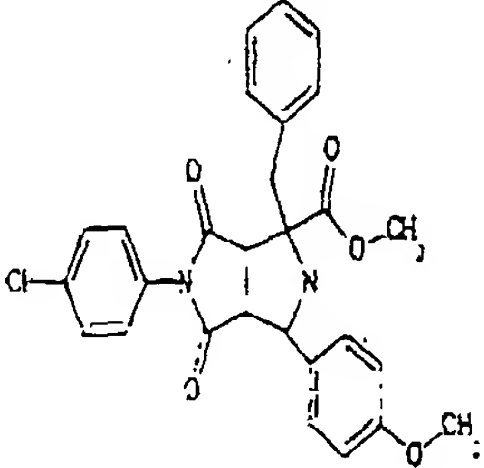
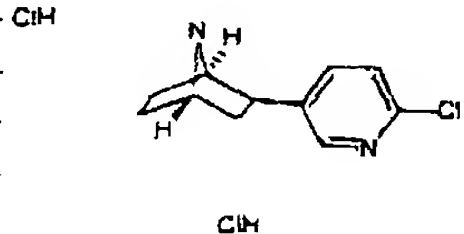
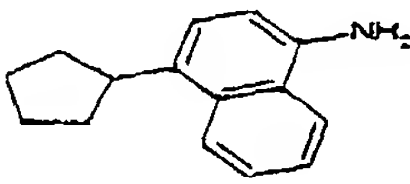
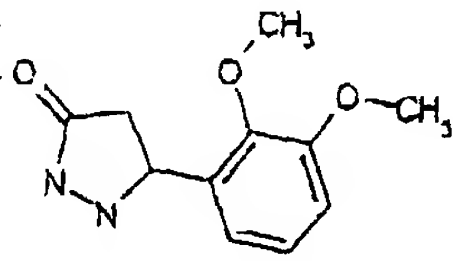
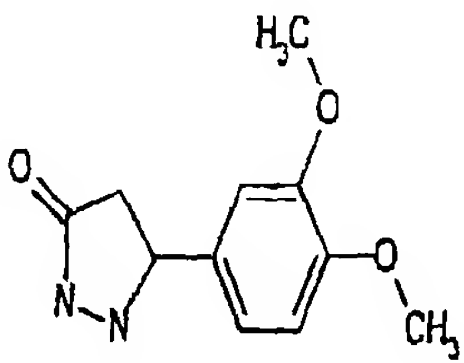
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	METHYL C-4-(4-METHOXYPHENYL)-2-BENZYL-7-PHENYL-6,8-DIOXO-3,7-DIAZABICYCLO[3.3.0]-OCTANE-R-2-CARBOXYLATE	MFCD00202518
	METHYL C-4-(4-METHOXYPHENYL)-2-BENZYL-7-(4-CHLOROPHENYL)-6,8-DIOXO-3,7-DIAZABICYCLO[3.3.0]-OCTANE-R-2-CARBOXYLATE	MFCD00202519
	(+/-)-EPIBATIDINE DIHYDROCHLORIDE	MFCD00210196
	4-CYCLOPENTYL-NAPHTHALEN-1-YLAMINE, HYDROCHLORIDE	MFCD00227852
	5-(2,3-DIMETHOXY-PHENYL)-PYRAZOLIDIN-3-ONE	MFCD00228403
	5-(3,4-DIMETHOXY-PHENYL)-PYRAZOLIDIN-3-ONE	MFCD00229211

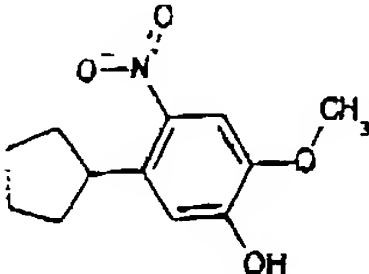
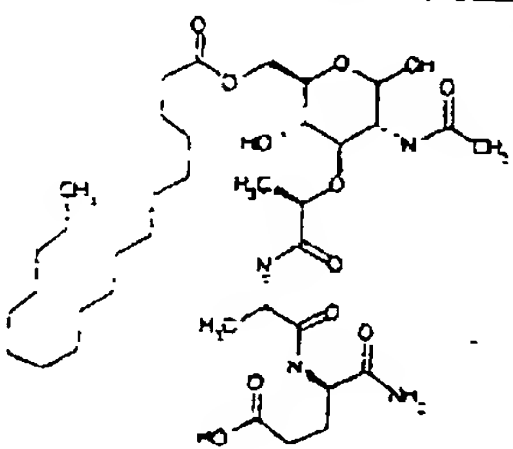
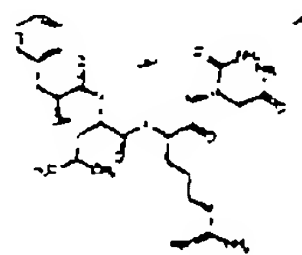
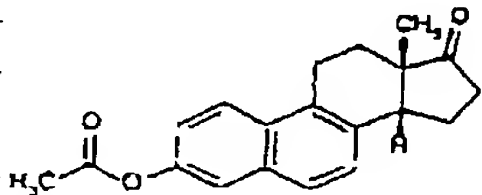
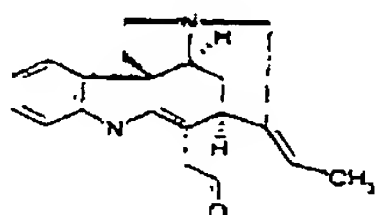
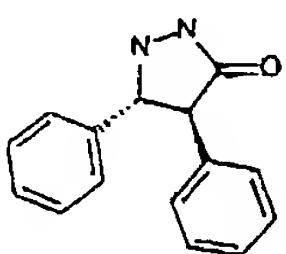
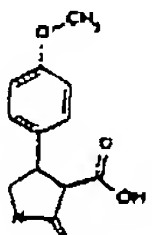
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	5-CYCLOPENTYL-2-METHOXY-4-NITRO-PHENOL	MFCD00230901
	AC-(6-O-STEAROYL)-MURAMYL-ALA-D-ISOGLUTAMINE	MFCD00236777
	ANTHO-RNAMIDE	MFCD00236789
	1,3,5(10),6,8(14BETA)-ESTRAPENTAEN-3-OL-17-ONE ACETATE	MFCD00271642
	NORFLUOROCURARINE	MFCD00274483
	4,5-DIPHENYLPYRAZOLIDIN-3-ONE	MFCD00277796
	4-(4-METHOXY-PHENYL)-2-OXO-PYRROLIDINE-3-CARBOXYLIC ACID	MFCD00297824

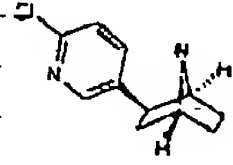
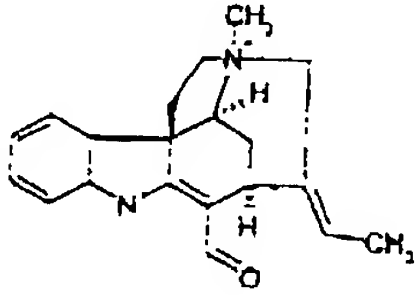
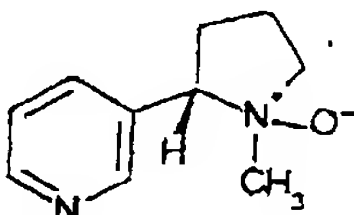
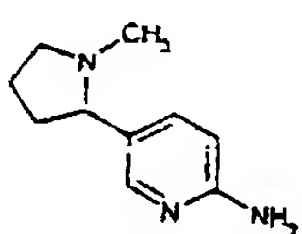
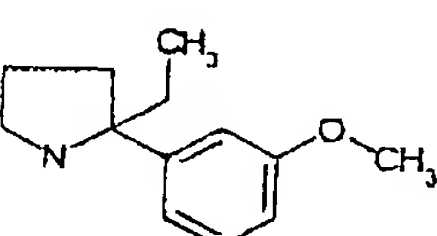
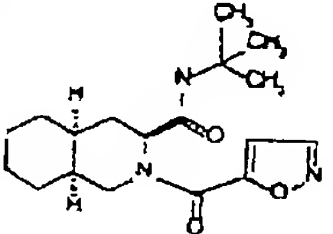
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	OH	(+)-EPIBATIDINE HYDROCHLORIDE	MFCD00467208
	Cl <sup>-</sup>	FLUOROCURARINE CHLORIDE	MFCD00467712
	CH <sub>3</sub>	(1'S,2'S)-NICOTINE 1'-OXIDE	MFCD00869528
	CH <sub>3</sub>	SPECS SPECS CIF7952	MFCD01114864
		2-ETHYL-2-(3-METHOXYPHENYL)PYRROLIDINE	MFCD01314146
		N-(TERT-BUTYL)-2-(ISOXAZOL-5-YLCARBONYL)DECAHYDROISOQUINOLINE-3-CARBOXAMIDE	MFCD01314517

**Norris, McLaughlin & Marcus, P.A.**

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New York, NY 10017

If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

#4

COMBINED DECLARATION AND POWER OF ATTORNEY FOR  
PATENT APPLICATION

Attorney Docket No.  
101195-73

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,  
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original,  
first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed  
and for which a patent is sought on the invention entitled

Agents for Treating Human Diseases, Especially for Treating Tumors Such as Colonic Cancers and  
Melanomas or for Regenerating Tissue and Promoting Hair Growth

the specification of which (check one)

\_\_\_\_\_ is attached hereto

☒ was filed on \_\_\_\_\_ 7 September 2000 \_\_\_\_\_

under Serial Number \_\_\_\_\_ PCT/DE00/03104 and was amended on \_\_\_\_\_  
(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification,  
including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in  
accordance with Title 37, Code of Federal Regulations, Section 1.56.

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign  
priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's  
certificate in respect of which such foreign priority rights are not claimed and which has a filing date before  
that of any application in respect of which such foreign priority benefits are claimed:

Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119
199 44 404.8	Germany	16 September 1999	YES: <input checked="" type="checkbox"/> NO: _____
			YES: _____ NO: _____
			YES: _____ NO: _____

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional  
application(s) listed below.

Application No.	Filing Date



Combined Declaration and Power of Attorney

Customer No. 27387

101195-73

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I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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**Kurt G. Briscoe (33,141) William C. Gerstenzang (27,552) Robert A. Hyde (46,354)**  
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	Post Office Address	City	State & ZIP/Country
<b>204</b>	Family Name	First Given Name	Second Given Name
	City of Residence	State or Foreign Country	Country of Citizenship
	Post Office Address	City	State & ZIP/Country

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101195-73  
Page 3

<b>205</b>	Family Name	First Given Name	Second Given Name
	City of Residence	State or Foreign Country	Country of Citizenship
	Post Office Address	City	State & ZIP/Country
<p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.</p>			
Signature of Inventor 201		<i>x W. Bidner</i>	Date <i>x April 11, 2002</i>
Signature of Inventor 202		<i>x Jan Kutz von Witz</i>	Date <i>x March 21, 2002</i>
Signature of Inventor 203			Date
Signature of Inventor 204			Date
Signature of Inventor 205			Date